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Procedures for Laboratory Diagnosis of TUBERCULOSIS

For Use in the Animal Disease Diagnostic Laboratories

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

PREFACE

The material in this manual has been prepared as a procedural outline for laboratories engaged in the diagnosis of tuberculosis. These methods were formulated by cooperative efforts of a committee on mycobacteriology with representatives from private, State, and Federal laboratories.

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PROCEDURES FOR LABORATORY DIAGNOSIS OF TUBERCULOSIS

INTRODUCTION

The methods presented below are intended as an outline of procedures for use in the examination of tissue specimens for evidence of tuberculosis. They are not intended to cover the subject in detail, as this information is readily available from other sources.

As the prevalence of tuberculosis in cattle has decreased to a very low level, the possibility of tuberculin sensitization with agents other than bovine tubercle bacilli has posed serious eradication problems in some areas. The methods presented in this outline are intended for use in the examination of tissue specimens in order to gather additional information on the cause of reactions due to infection with bovine, avian, or human type of tubercle bacilli, or to other related organisms.

The possibilities of laboratory infection are greatly increased in the tuberculosis laboratory today due to the increased handling of viable organisms. Today's methods of handling, culturing, and conducting animal studies and chemical tests all favor the possibility of human exposure. It is necessary for laboratorians to use utmost care to avoid the exposure of themselves and others to infection with the tubercle bacillus. Proper equipment does much to minimize the chance of exposure; however, the laboratorian himself remains the most essential link of any safety program.

PERSONNEL AND SUPPLIES FOR COLLECTING SPECIMENS

A properly trained individual should be employed in the collection, handling, packing, and delivery of specimens to the laboratory. This individual should be instructed in the proper techniques of selection and collection, and impressed with their importance in yielding maximum information. Clean knives, scissors, forceps, 10 percent aqueous buffered formalin, household borax, sterile stoppered bottles, and labels are essential for proper collection of specimens.

COLLECTION OF SPECIMENS

Macroscopic Lesions

Records are very important in making collections. Data should be recorded for each animal and each specimen identified by labeling. Sterile knives and forceps should be used between tissues to prevent contamination. If this is not possible, clean knives and forceps should be submerged in boiling water for 30 seconds.

All suspicious lesions of any tissue should be collected. Do not incise specimens any more than necessary. When multiple lesions are found, one or more should be submitted unincised. Specimens should be accurately identified, not contaminated after collection, and kept cool.

Single specimens smaller than a small marble should not be divided in the plant for a separate histopathological specimen. The entire specimen should be packed in household borax and properly labeled. Histopathological specimens should be taken from multiple or medium to large-sized lesions. These lesions are deposited immediately in a wide-mouthed bottle containing 10 times as much 10-percent aqueous formalin as tissue. The bottle should be properly labeled and tightly stoppered. Care should be taken to prevent contamination of all containers with specimen material. The bottle containing the specimen should be dipped in a disinfectant solution before final packing and dispatching to the laboratory.

No Macroscopic Lesions

Where macroscopic lesions are not detected in reacting animals, consideration may be given to collecting representative specimens (preferably unincised) lymph nodes. Each group of nodes should be identified separately at the time of collection. In certain herds and areas there may be occasions where it is desirable to collect additional tissues. The specimens should then be packed in household borax for shipment to the laboratory.

LABORATORY PROCEDURES

If the specimen is small and the tissue limited, first consideration should be given to smears, cultural procedures, and animal inoculations. Specimen material should then be collected from small specimens for histopathologic examination and be deposited in 10-percent aqueous buffered formalin after removal of borax. If the specimen is large, smears, animal inoculations, and media inoculations should be conducted with the tissues supplied. Tissues for histopathological examination from such specimens will already have been taken at the plant. To prevent contamination of specimens, extreme care should be exercised in the laboratory as well as in the plant.

Preparation of Specimens

Specimens should be placed in boiling water for a period of 5 seconds before processing, to lower bacterial flora on their outer surfaces and to minimize contamination. In the absence of lesions, the specimen is placed in a sterile mortar and is cut into small pieces with sterile scissors. It is then emulsified with a piece of sterile copper wire gauze and a pestle, small amounts of sterile broth being added until a thick suspension is obtained. Homogenizers may be used in lieu of mortar and pestle, if the tissue is incised and care taken to avoid human exposure from the homogenizer.

Only in those incidences when it is suspected that specimens are contaminated with extraneous organisms lethal to test animals should the specimens be treated with an equal amount of 1 percent sodium hydroxide for 15 minutes. After centrifugation at 3,000 r.p.m. for 15 minutes, the supernatant fluid is decanted and the sediment neutralized by 0.2 N hydrocholoric acid. The neutrality of the specimen then can be tested by a suitable indicator.

Animal Inoculations

All animals should be from known clean stock and should be tuberculin-tested prior to their use as inoculation animals.

A dosage of 2 to 5 ml. of the triturated tissue suspension should be used for each animal inoculation. Guinea pigs and rabbits should, in all cases, be inoculated—at least two with each specimen. Guinea pigs are inoculated subcutaneously in divided doses into the sternal region. Rabbits and chickens are inoculated subcutaneously and intravenously respectively.

Inoculated animals should be tuberculin tested at 6 weeks post-inoculation. If the test is positive, sacrifice one animal. The other animal should not be sacrificed unless lesions of tuberculosis are found in the first animal. If the 6-week post-inoculation test is negative, they should again be tested at 12-week post-inoculation. All animals may be sacrificed and cultured following this test. Any animals that die prior to this time are necropsied and cultured.

Smears

Smears may be prepared directly from the ground tissue suspensions or from the sediment in cases where sodium hydroxide treatment is used. With some specimens direct smears may be taken from the diseased tissues. By use of a sterile wire loop, several loopsful of the sediment are placed on a slide and a smear is made by rubbing the material with another slide. Broth may be used to obtain better fixation. After the smear has dried in the air, it is passed through a low flame two or three times or exposed to a heating tray for fixation. Excessive heat is to be avoided as the staining qualities of tubercle bacilli may be affected by high temperature. The specimen is then stained by the Ziehl-Neelsen technique. At least 100 fields should be examined before the slide is considered negative.

Inoculation of Culture Media

Material for culture inoculation should be taken directly from the sediment suspended in a small amount of sterile broth. Sterile swabs or glass spatulas may be used to inoculate any of the suggested media below. The material is spread evenly over the surface of at least four slants of solidified media. Of the media used, at least half of the tubes should be without glycerol. Some of these media are available commercially in sealed tubes. It is preferable, although not necessary, to keep the tubes in a slanting position for 24 hours to allow even distribution of the colonies. Tubes are then incubated at 37° C. for 8 to 12 weeks, examinations being made at weekly intervals for evidence of growth. Additional cultures may be incubated at 30° C. and others may be incubated under CO_2 tension as growth may be accelerated by CO_2 tension. Other special media may be used if desired.

TYPING CULTURES OF M. TUBERCULOSIS

This may be done by animal inoculation and growth studies on special culture media. Reference may be made to standard textbooks on this subject.

SUGGESTED MEDIA

- Lowenstein-Jensen medium
- Stonebrink medium
- 7H10 medium (under 10 percent CO_2)
- Dubo's agar
- Tarshis medium
- Herrold's medium

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